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Methodology of stem water potential measurement on hedgerow olive orchards

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Abstract

Aim of study: To evaluate the effect of leaf covering, leaf position, leaf age, time and sample size in measurements of stem water potential (Ψstem) in olive hedgerow orchards.

Area of study: The experimental orchards were located in the Centre of Spain (Toledo).

Material and methods: Midday Ψstem was measured using a pressure chamber in two super-intensive olive hedgerow orchards subjected to various water status.

Main results: Measurements were taken at solar noon on shaded leaves at mid canopy height following at least 1 hour of covering. Such measurements on 5 trees were sufficient to define the water status of individual homogeneous irrigation blocks. This combination of techniques is essential for repeatable measurements of Ψstem required to establish critical irrigation points and manage deficit irrigation strategies seeking to control vegetative growth and reduce water use with minimal effect on oil yield in olive hedgerow orchards and maximum oil quality. Part of our results weren't previously reported: 1) Covering leaves with aluminium doesn't completely stop transpiration and leaves must be covered and located in the shaded part of the canopy. 2) Ψstem depends on leaf height on olive hedgerow. 3) Ψstem of young leaves was less variable than in older ones. 4) Ψstem at solar noon presented larger differences with previous hours than later.

Research highlights: Leaves for measurements of stem water potential must be previously covered and located in the middle height of the shaded part of the canopy.

Additional key words: water status; *Olea europaea*; irrigation management; water stress.

Abbreviation used: CV (coefficient of variation); VPD (vapour pressure deficit); Ψleaf (leaf water potential); Ψstem (stem water potential).

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Introduction

Since they first appeared in Spain in the middle of the 90s, high density olive (*Olea europaea* L.) hedgerow orchards, with tree densities greater than 1500 trees/ha, have undergone a great expansion in the last decades, up to 100,000 ha in 2010 (Rius & Lacarte, 2010) and 500,000 ha in 2017 (Vilar & Pereira, 2017). The objective of this system is to facilitate mechanical management of pruning and harvesting. It is a revolution in olive management relative to traditional low-density orchards (100-300 trees ha^{-1}) grown mostly without irrigation under low rainfall conditions (Rallo et al., 2013; Connor et al., 2014). Most irrigation experiments have been carried out in low-density orchards (Fernández & Moreno, 1999) but, because of different orchard structure, high intensive orchards require different irrigation management (Trentacoste et al., 2015). Furthermore, in many olive growing areas, the price of irrigation is expensive and water is scarce. Then deficit irrigation strategies for optimal use are needed. Regulated deficit irrigation (RDI) reduces irrigation during the phenological stages resistant to drought (Chalmers et al., 1986) but requires monitoring of the tree water status and determination of threshold values.

Leaf water potential (Ψleaf) measured with a pressure chamber is the most commonly used measurement of plant water status for irrigation programming (Turner & Long, 1980; Fernández, 2017). Measurements of Ψleaf made on sunlit leaves are variable because they depend upon leaf conductance (Naor et al., 2001) but have the advantage that covering is not required. Consequently, measurement of shaded leaves close to the trunk, termed stem water potential (Ψstem), is considered the standard measurement to determine tree water status (Begg & Turner, 1970; Shackel et al., 1997). These values are less variable and more stable than Ψleaf and more clearly related with soil water content (McCutchan & Shackel, 1992; Santesteban et al., 2019). At solar noon, Ψstem values reach a minimum value. Shackel et al. (1997) observed that midday Ψstem can be a guide for irrigation programming in several studies of deficit irrigation in pear, almond, plum and cherry production. Marino et al. (2018) determined the physiological response of olive to water stress determining different levels: no stress (Ψstem above -2.0 MPa), moderate stress (between -2.0 and -3.5 MPa) and high stress (below -3.5 MPa). In a hedgerow olive orchard, Ahumada-Orellana et al. (2019) also observed mild or absent water stress above -2.0 MPa. To maintain high leaf turgor, Fernández et al. (2011) and Padilla-Díaz et al. (2016) recommend maintaining Ψstem above -1.7 MPa.

In most fruit trees, Ψstem varies with the evaporative conditions (Naor et al., 2006), meanwhile in olive the relationship between Ψstem and vapour pressure deficit is weak (Moriana & Fereres, 2003). This aspect of olive tree increases the interest of using this measurement for olive irrigation scheduling. In recent years, threshold values of Ψstem have been determined at various phenological stages with corresponding impacts on vegetative growth, production and oil quality. Trentacoste et al. (2019) observed that maintaining Ψstem between -1.2 MPa and -1.7 MPa during spring hedge dimensions were controlled. A similar value (-1.2 MPa) was recommended by Moriana et al. (2012) before the beginning of massive pit hardening. By contrast, Ψstem can fall to -2.9 MPa during pit hardening (July in the North hemisphere) without effect on production (Gomez-del-Campo, 2013) but with increased phenolic content of oil (Gomez-del-Campo & García, 2013; Gucci et al., 2019). Later in August, and during the phase of oil synthesis (late summer and autumn), Ψstem should be maintained above -2.21 MPa for high production (Hueso et al., 2019) and high phenolic content (García et al., 2020).

Water potential depends on soil water content but also on tree architecture (García-Tejera et al., 2021) and on the resistance to water movement associated to differences in structure between traditional vase canopies and hedgerows. Some questions arise when Ψstem is measured in olive hedgerows. The low hydraulic conductance of olive (Fernandez & Moreno, 1999) may affect the measured leaves according to their position in a large hedgerow as was previously observed in tobacco plants (Begg & Turner, 1970), although in grapevine Chone et al. (2001) did not observe large differences between measurements of leaves 1 m apart. The measurement of Ψstem is made on covered leaves so a question arises of its importance within dense hedgerows. Additionally, the duration of covering has been variable in practice. Naor et al. (2006) covered shoots for 90 minutes, while Begg & Turner (1970) covered tobacco leaves the afternoon prior to measurement. In grapevine, Chone et al. (2001) did not observe differences in covering leaves for 1, 2 or 6 hours. At midday, Ψstem is stable and measurements are less variable, even so a short period of covering reduces the number of plots that can be monitored. Olive is a perennial tree with leaves that persist for more than 2 years. The age of the stem determines leaf hydraulic properties (Fernández et al., 1997) so it may also affect Ψstem values.

The use of Ψstem for irrigation scheduling of commercial orchard requires the minimum representative number of samples that will depend on the sensitivity and variability of the measurements (Naor & Cohen, 2003; Intrigglio & Castel, 2004). Naor et al. (2006) observed that the reasonable sample size n = 7 allowed a variation of the Ψstem ± 0.15 MPa in an apple orchard.

For all these reasons, the present work performed various experiments to identify the variability introduced in Ψstem measurements by leaf covering, leaf position, leaf age, time of measurement and sample size. The objective was to develop a protocol for Ψstem measurements in olive hedgerow orchards.

Material and methods

Experimental sites

In 2016, 2017 and 2018, five experiments were conducted in two hedgerow olive cv. Arbequina orchards. Both orchards are located in the center plateau of Castilla in Toledo, Spain, characterized by low rainfall (annual average of 395 mm), a high evaporation (annual average of 1,180 mm) and a long period with frosts (November to March), corresponding to Mediterranean climate. A weather station located 100 m far from the experimental trees provided information about daily rainfall, wind direction and speed, temperature and humidity.

The 20-ha Orchard A is located in La Puebla de Montalbán, Toledo (Lat: 39º48'19" N; Long: 4º27'5"; 511 masl), with an average slope of 5%. It was planted in 1997 at 4 × 2 m (1250 trees ha^{-1}) with rows in a NW-SE orientation. The soil is Haploxeralf typic with an effective rooting depth of 0.60 m, each layer being 0.20 m deep. The texture is clay loam, clay loam and sandy clay loam for the three horizons in sequence. Irrigation was applied by 2.2 L/h drippers spaced 0.60 m. The 13-ha Orchard B is located in El Viso de San Juan (Lat: 40º8'0.07" N; Long: 4º1'27.71"; 566 masl) with an average slope of 3%. It was planted in 2010 at 6 × 1.5 m (1027 trees ha^{-1}) with rows oriented NW-SE. The soil is calcic (Haploxeralf calcium) with an effective rooting depth of 0.62 m, 0.28 m in the first horizon and 0.34 m the second, with sandy loam and clay loam textures, respectively. Irrigation was applied by 3.8 L/h drippers spaced 0.75 m.

In both orchards, weeds were controlled with a non-residual herbicide and fertilizers were applied during the season according to leaf nutrient analyses made each year in July. Mechanical and manual pruning for Orchard A and manual pruning for Orchard B, kept the hedgerow dimensions at 2.1 m high and 1.1 m wide and 2.0 m high and 1.0 m wide, respectively.

Experiments

Stem water potential (Ψstem, MPa) was measured on apical shoots with 4 leaves at midday (11:30–12:30 solar time) with a pressure chamber (Soil Moisture Equip., Santa Barbara, CA, USA). Pressure increase rate inside the instrument was ca. 0.03 MPa/s (Naor et al., 2006). Sample was excised and later recutted before measurement (Levin, 2019). One apical shoot per tree was measured. Repetition trees were located in the same irrigation sector with similar growing and productive characteristics. In order to establish a wide range of Ψstem, irrigation was stopped after large water applications and measurements started the day later.

— 1. Covered leaves (Exps. 1 and 2)

In 2016, two experiments were carried out in Orchard A to answer three questions related to covering stem apices with aluminum bags prior to measurement of Ψstem. Is covering necessary? What duration of covering is required? Should the leaves for measurement be located in the shaded part of the canopy?

Irrigation was stopped after large water applications on 18/07/2016 and 31/08/2016. The days of measurements were 19, 21, 22 and 23/07/2016 and 1, 3, 5, 6, and 7/09/2016.

In one experiment (Exp. 1), midday Ψstem was measured on apical shoots with 4 leaves in the shaded part of the canopy that had been uncovered, covered during 1 and 2 h on the same 10 trees on 4 days in July and 5 days in September. Trees were located in the same line in an homogenous soil.

In another experiment (Exp. 2), Ψstem was measured on apical shoots covered for 1 hour on both the sunny and shaded sides of a hedgerow. In all cases, the measured apical shoots were less than 1-yr-old and were located at middle height of the canopy (0.85-1.7 m) with each measurement made on individual trees.

— 2. Height within hedgerow (Exp. 3)

On the same days and in the same orchard of covered leaves experiments (Exp. 1 and 2), measurements of midday Ψstem were made on 1-yr-old shoots with 4 leaves, previously covered for 2 h, at three heights, H0 (0.40–0.85 m), H1 (0.85–1.7 m) and H2 (1.7–2.1 m), on each ten trees.

— 3. Leaf age (Exp. 4)

In 2017 in Orchard A, irrigation was stopped on 10 trees after a large application on 18/07/2017. Ψstem was measured at midday after 1-hour covering on 19, 21, 24, 26 and 28/07/2017 on three apical shoots per tree with 4 leaves of 1, 2 and 3-yr old, respectively, located in the shaded, middle part of the hedgerow (0.85–1.70 m). The youngest leaves in the stem were developed before the last year, 2-yr old correspond to the part of the stem with fruits and 3-yr old leaves are located below fruit position.

— 4. Hour of measurement (Exp. 5)

On the same days and trees of Exp. 4, Ψstem was measured at 10:00, 12:00 and 14:00 solar time on 1-yr-old shoots with 4 leaves, previously covered for 1 h and located at 0.85-1.70 m height.

— 5. Experiment of sample size of tree number (Exp. 6)

In 2018 in Orchard B, irrigation was stopped on 30/07/2018 in three sectors each of 3 ha. Within each sector soil characteristics were homogeneous. Midday Ψstem was measured in an apical shoot with 4 leaves in 15 trees in each sector on 31/07/2018 and 2/08/2018, 4/08/2018, 6/08/2018 and 8/08/2018. Measurements were made on apical shoots of 1-yr old leaves, previously covered for 1 h, and located in the shaded side of the hedgerow at 0.85-1.70 m height.

Summarizing, Exps. 1, 2 and 3: Orchard A, irrigation stopped after large water applications 18/07/2016 and 31/08/2016, measurements on 19/07/2016, 21/07/2016, 22/07/2016 and 23/07/2016 and 1/09/2016, 3/09/2016, 5/09/2016, 6/09/2016, and 7/09/2016. Exps. 4 and 5: Orchard A, irrigation stopped 18/07/2017, measurements on 19/07/2017, 21/07/2017, 24/07/2017, 26/07/2017 and 28/07/2017. Exp. 6: Orchard B, irrigation stopped 30/07/2018, measurements on 31/07/2018 and 2/08/2018, 4/08/2018, 6/08/2018 and 8/08/2018.

Statistical analysis

A statistical summary was obtained for each data set obtained from each experiment. So the mean and the coefficient of variation, CV% = (standard deviation/mean) × 100, was calculated as relative measure of spread.

Table 1. Daily reference evapotranspiration (ET_0), vapour pressure deficit (VPD) and maximum and minimum temperature during the days of measurements and mean monthly values.

Date	ET_0 (mm)	VPD (kPa)	T max (ºC)	T min (ºC)
23/08/2015	4.5	1.50	33.8	13.9
06/09/2015	4.1	2.29	33.0	16.1
29/09/2015	3.7	1.21	29.1	11.8
31/10/2015	1.3	0.28	22.1	7.0
19/07/2016	6.3	2.69	40.8	15.5
21/07/2016	6.9	2.22	36.8	14.9
22/07/2016	6.4	2.19	34.9	14.5
23/07/2016	6.8	2.12	33.4	19.2
01/09/2016	5.7	1.92	35.8	16.0
03/09/2016	4.7	1.96	37.5	14.3
05/09/2016	4.9	2.57	39.9	17.5
06/09/2016	4.7	2.50	41.3	16.3
07/09/2016	5.8	2.55	40.1	16.4
19/07/2017	7.1	2.03	33.6	18.8
21/07/2017	6.7	1.88	32.7	14.5
24/07/2017	6.4	2.28	34.5	16.5
26/07/2017	6.0	2.12	36.1	14.0
28/07/2017	6.7	2.30	39.0	15.6
31/07/2018	5.5	2.84	39.9	16.1
02/08/2018	6.1	3.46	43.6	21.3
04/08/2018	5.5	3.21	42.3	17.5
06/08/2018	6.1	3.77	42.8	21.8
08/08/2018	4.8	2.77	40.2	14.5
August 2015	147.2	1.84	38.9	9.0
Sept 2015	114.6	1.41	37.4	6.8
Oct 2015	64.6	0.77	33.6	0.0
July 2016	199.4	2.19	40.8	16.8
Sept 2016	120.4	1.39	41.3	11.7
July 2017	195.3	1.91	42.6	9.5
July 2018	185.7	2.18	37.1	13.8
August 2018	168.6	2.51	42.5	14.4

The software used for statistical analysis was Infostat version 1.5 (National University, Córdoba, Argentina) and Statgraphics Centurion v 18.1.12. (Statgraphics Technologies, Inc., The Plains, VA, USA). The LSD test was calculated to detect significant differences between treatments.

The statistical optimal sample size (n) (Exp. 6) was determined using the known expression:

$$n=\left(\frac{z_{\alpha/2}*S}{e}\right)^2=\left(\frac{z_{\alpha/2}*100(S/\bar{x})}{100(e/\bar{x})}\right)^2=\left(\frac{z_{\alpha/2}*CV}{e_r}\right)^2$$

where S is the standard deviation; e is the absolute error (1 MPa, in our case); $z_{\alpha/2}$ =1.96 (95% percentile of the Normal distribution); $\bar{x}$, is the data mean; CV % is the data coefficient of variation; and e_r is the relative error (1.2% in this case). The third expression was the one used to calculate the sample size from data of last experiment.

Results

The environmental conditions and climatic data of the days of measurements are summarized in Table 1. In 2016, 2017 and 2018 there were days of high evaporative demand (ETo > 4.7 mm/day). The maximum was 7.1 mm (19/07/2017) and the minimum of 4.7 mm (6/09/2016).

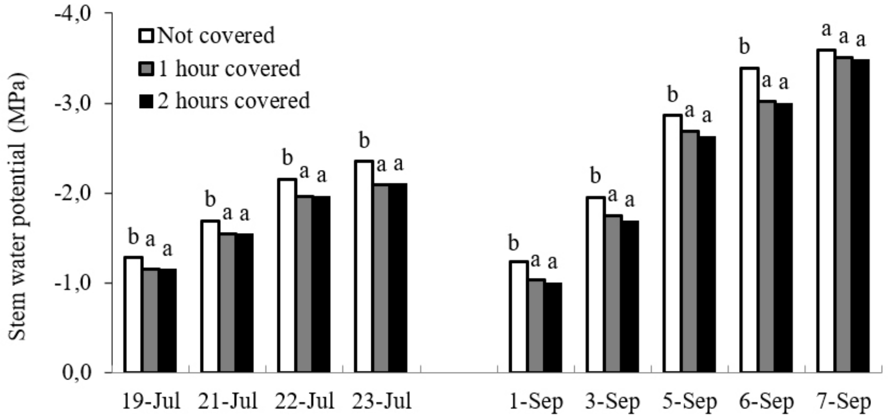

Figure 1. Experiment 1: Difference in midday stem water potential measured in leaves not covered, and previously covered during 1 and 2 hours located in the shade and middle part of the hedgerow (0.85-1.70 m). Last irrigations were applied on 18th July and 31st August 2016. Different letters indicate significant differences ($p \leq 0.05$) within covering treatments according to the LSD test.

The highest daily maximum temperature was 43.6 ºC (2/08/2018). The maximum value of daily vapour pressure deficit (VPD) was 3.77 kPa (6/08/2018).

The monthly data reveal the warm and dry summer months (July and August) with ETo higher to 150 mm, and the mild climate in autumn (October- 64.6 mm).

1. Covered-leaf experiments (Exps. 1 and 2)

Ψstem of covered leaves was significantly lower than those leaves that were uncovered and located in the shade (Fig. 1) except on the most stressed day (7/09/2016) when Ψstem reached -3.55 MPa. Overall, the average Ψstem from 10 trees over 9 days was -2.08 and -2.28 MPa for

Table 2. Values of sample size and average values of water potential (Ψ, MPa), coefficient of variation (CV, %), standard error and statistical p-value of ANOVA of treatments evaluated in the five experiments of Ψstem methodology: covered, location and age of leaves, and hour of measuring (n=10).

Experiments	n	Ψ [1]	CV	SE	p-value
1. Leaves:					
Not covered	90	-2.28 b	3.89	0.03	0.10
1 hour covered	90	-2.08 a	2.98	0.02	
2 hours covered	90	-2.06 a	3.03	0.02	
2. Leaves located:					
in the shade	90	-2.13 a	3.30	0.01	0.01
in the sun	90	-2.54 b	3.38	0.01	
3. Hedgerow height position:					
H0 (0.40-0.85m)	90	-2.06 a	2.71	0.01	0.08
H1 (0.85-1.70m)	90	-2.13 b	3.71	0.01	
H2 (1.70-2.10m)	90	-2.33 c	3.71	0.02	
4. Age of leaves:					
1 yr old	40	-2.81	3.74	0.03	0.99
2 yrs old	40	-2.80	4.39	0.04	
3 yrs old	40	-2.81	4.18	0.04	
5. Hour of measurement:					
10:00 solar hour	40	-2.38 a	4.19	0.04	0.06
12:00	40	-2.81 b	4.28	0.05	
14:00	40	-2.74 c	3.58	0.04	

[1] Different letters within the column indicate significant differences between treatments according to the LSD test ($p<0.1$).

Table 3. Experiment 2: Differences of midday stem water potential (Ψstem, MPa) measured in leaves located in different sides of the hedgerow (shade and sunny) previously covered during 1 hour and located in the middle part of the hedgerow (0.85-1.70 m). CV = coefficient of variation (%), standard error (SE) and statistical p-value of ANOVA. Number of repetitions: n=10.

Day of measurement	Days since last irrigation	Covered shade			Covered sunny			p-value
		Ψstem	CV	SE	Ψstem	CV	SE	
19/07/2016	1	-1.19 a	6	0.02	-1.53 b	5	0.03	0.01
21/07/2016	3	-1.62 a	2	0.01	-2.09 b	5	0.03	0.01
22/07/2016	4	-2.03 a	4	0.03	-2.56 b	3	0.02	0.01
23/07/2016	5	-2.16 a	3	0.02	-2.66 b	3	0.02	0.01
01/09/2016	1	-1.05 a	4	0.01	-1.40 b	4	0.02	0.01
03/09/2016	3	-1.79 a	3	0.02	-2.20 b	5	0.03	0.01
05/09/2016	5	-2.70 a	3	0.03	-3.01 b	3	0.03	0.01
06/09/2016	6	-3.09 a	2	0.02	-3.60 b	2	0.02	0.01
07/09/2016	7	-3.58 a	2	0.03	-3.84 b	2	0.02	0.01

Different letters within the columns indicate significant differences between treatments according to the LSD test $p<0.1$.

covered and uncovered shoots, respectively (Fig. 1). Lower variability was observed in the covered (2.98%) than the uncovered shoots (3.89%) (Table 2) and in both treatments, variability decreased with stress. The differences between covered and uncovered leaves were reduced under more stressed conditions (Fig. 2A; $R^2 = 0.49$). The duration of covering (1 or 2 h) did not significantly affect Ψstem in either average or variability (Fig. 1; Table 2). The average value of Ψstem was -2.08 MPa and -2.06 for 1 h and 2 h covering, respectively.

The position of the covered leaves in the shaded and sunny sides of the canopy significantly affected Ψstem (Table 3) and caused differences of 34% (Fig. 2B). The average value of Ψstem was -2.13 MPa for leaves located in the shaded side and -2.54 MPa for those exposed to the sun (Table 3). Variability was slightly higher in leaves exposed to the sun than in the shadow (Table 2). The differences between covered and uncovered shoots were reduced under more stressed conditions (Fig. 2B; $R^2 = 0.88$).

2. Hedgerow height-position experiment (Exp. 3)

Ψstem was significantly affected by height position in the hedgerow (Fig. 3). In most measurements Ψstem was higher when measured in the lower part of the canopy H0 (0–0.85 m) than in the middle part H1 (0.85–1.7 m) and in the higher part H2 (1.7–2.1 m). The average values of Ψstem were -2.06, -2.13 and -2.33 MPa for H0 (0.6 m height), H1 (1.3 m) and H2 (1.9 m) respectively (Table 2). The difference between heights were significantly different at $p<0.08$ (Table 2). The average difference between H0 and H1 is 0.07 MPa and between H0 and H2 is 0.24 MPa. The increase in height from H0 until H2 produced a reduction of 0.21 MPa/m, but this increase was lower in the first 0.65 m (H0-H1) (0.11 MPa/m) than in the next 0.62 m (H1-H2) (0.31 MPa/m). Variability was lower in H0 (2.71%) but was similar between H1 and H2 (3.71%) (Table 2). The differences between heights were reduced under most stressed conditions (Fig. 2C, $R^2 = 0.58$; Fig. 2D, $R^2 = 0.44$).

3. Leaf-age experiment (Exp. 4)

The average values of Ψstem were similar ($p>0.99$) at -2.81, -2.80 and -2.81 MPa for 1, 2 and 3 yr-old leaves, respectively (Tables 2 and 4), consistently on all measurement days. The mean CV of Ψstem of 1-yr-old leaves (3.74 %) was lower than in leaves of 2- and 3-yr-old leaves (4.39 and 4.18%).

4. Time of measurement (Exp. 5)

Ψstem was significantly affected by the time of measurement (10, 12 and 14 solar hours) (Fig. 4). Measurements at 10:00 solar hours (mean value of -2.38 MPa) were significantly higher than those at 12:00 (-2.81 MPa) and 14:00 hours (-2.74 MPa) at $p<0.06$ (Table 2). Under low stress conditions (>-2.20 MPa) Ψstem was not significantly different between measurements at 12:00 or 14:00. But under high stress the lowest values were achieved at 12:00. Lower variability was obtained at 14:00 hours (3.6%) than at the other hours (4.2%). The differences between times of measurement were not related with the stress conditions (Fig. 2E and 2F).

5. Sample-size experiment (Exp. 6)

The mean statistical optimal sample size required for the three sectors evaluated was 5 trees sampling 1 apical shoot per tree with a mean CV of 3% (Table 5). At the beginning of the experiment (31/07/2018), Ψstem values were -2.30, -2.37 and -2.36 MPa, falling to -4.64, -4.80 and -4.85 MPa for sectors 1, 2 and 3, respectively, during the drying period.

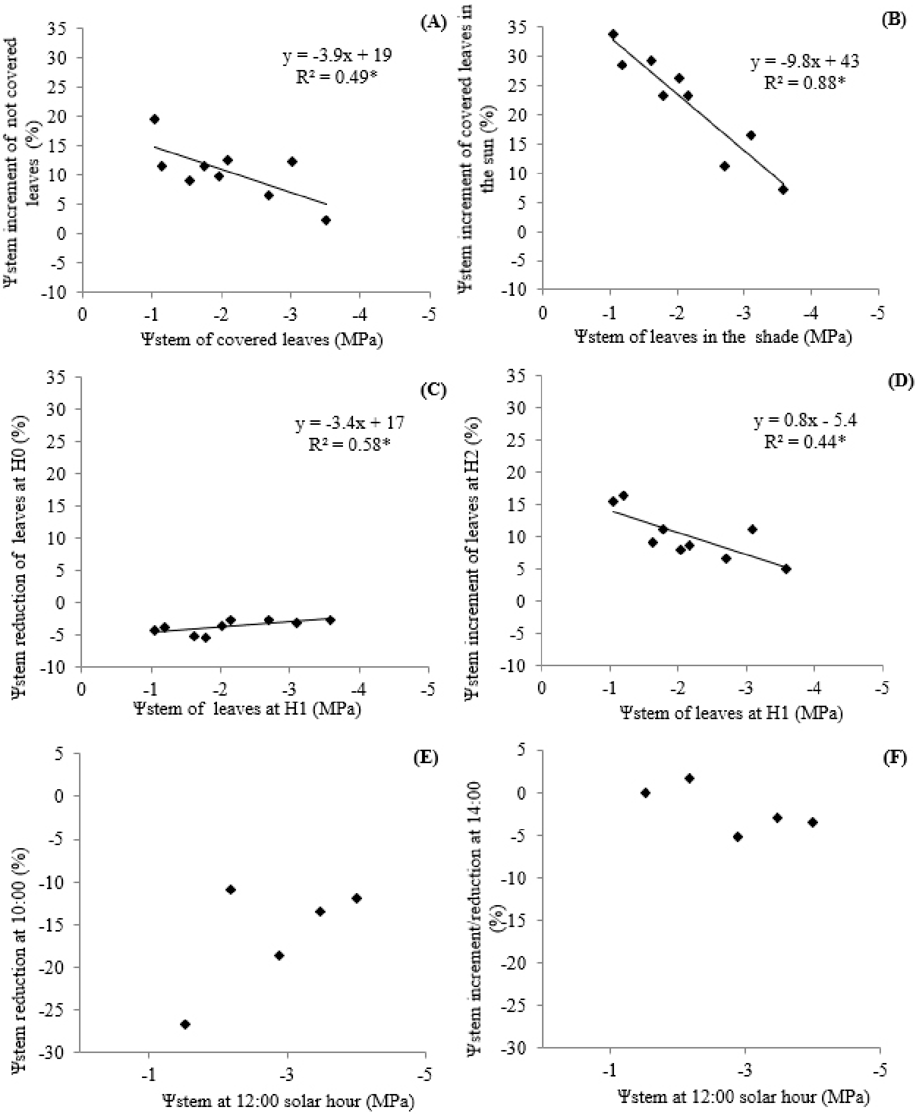

Figure 2. Relationship of the increment or reduction of Ψ (expressed as % of reference Ψstem) in the different experiments: Exp. 1, not covered leaves (A); Exp. 2, covered leaves located in the sun (B); Exp. 3, leaves located at H0=0.40-0.85 m (C); Exp. 3: H2=1.70-2.10 m (D); Exp. 5, measured at 10:00 (E); and Exp. 5, 14:00 (F) solar time and the reference Ψstem. Reference Ψstem was determined at solar noon in leaves previously covered during 1 hour, located in the shade at H1=0.85-1.70 m. * significant relationship at $p<0.05$.

Discussion

Results of our experiment provide guidance for Ψstem measurement in olive hedgerows for both experimental and management purposes. They can be used to answer some questions that arise when implementing Ψstem for irrigation management of commercial hedgerow olive orchards: Is it really necessary to cover the leaves before measurement or is it enough with selecting shaded leaves? Do I need to cover the leaves for more than 1 hour? Can I measure in sunny leaves previously covered? Can I measure in old leaves? In a 2 m hedgerow, will the height of the leaves determine the value of Ψstem? Can I measure some time before or after solar midday? How many samples are representative for an irrigation plot?

The experimental orchards were located in the southern plateau of Castilla (516 masl) in the centre of Spain that is characterized by dry and warm summers (Table 1) corresponding to the Mediterranean climate by Köppen (1918).

Ψstem measurements were conducted under different phenological olive stages in the middle of the irrigation season (July, August and September) under high evaporative conditions (ETo=4.7–7.1 mm/day). Results of the methodologies of Ψstem measurements that were evaluated depended on the range of Ψstem experienced by the orchards. Each methodology was evaluated during

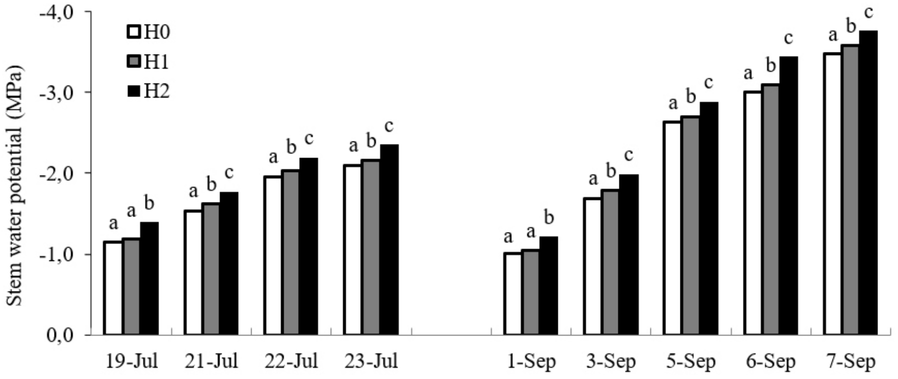

Figure 3. Experiment 3: Differences in midday stem water potential measured in leaves located at different heights of hedgerow orchard (H0=040-085 m, H1=085-170 m, H2=170-210 m). Leaves were covered during 2 hours. Last irrigations were applied on 18th July and 31st August 2016. Different letters indicate significant differences (p≤005) within leaf height positions according to the LSD test.

consecutive days after irrigation. In this case, climatic conditions, soil characteristics and irrigation practice produced a daily range of Ψstem values in excess of 2 MPa. Values evolved from non-water stress conditions (above -2.0 MPa) to high stress (below -3.5 MPa) following the stress threshold values of Ψstem described in Marino et al. (2018). In most of the experiments, differences between treatments were reduced under the most stressed conditions (Fig. 2). It is also noteworthy that the results of individual experiments, repeated on different days, were similar. The number of repetitions (n=10) was larger than in other methodological experiments (Levin, 2019).

Ψstem is used for irrigation in fruit orchards because values are reliable and repeatable (Fulton et al., 2001). In olive orchards its use is increasing given that it provides plant physiological data, which together with soil and climatic conditions contribute to optimal water use. In the last years, an important irrigated surface is being cultivated with hedgerow olive orchards (Rallo et al., 2013; Connor et al., 2014).

Ψstem was significantly higher (by 0.20 MPa) and was less variable when leaves were covered (Fig. 1, Fig 2A and Table 2) rather than uncovered, even when they were in the shade. Under well hydrated conditions differences reach 15% of covered ones. The stomatal conductance of uncovered leaves interacts with Ψ (Shackel et al., 1997) because stomata remained open (Gregoriou et al., 2007) thereby reducing Ψstem. In any case, these results indicate that shoots should be covered even if they are located in the shadowed part of the canopy. The reduction of the transpiration and consequent CV of Ψstem was reduced in nearly 1% (Table 2). The differences between covered and uncovered leaves were reduced under more stressed conditions (Fig. 2A, $R^2 = 0.49$), indicating that under high stress conditions transpiration was nearly stopped and covering is not necessary.

The use of Ψstem for irrigation management of commercial orchards should consider these results. Although it is cumbersome to cover the shoots 1 hour before the measurement, the references of Ψstem for irrigation management were obtained under uniform conditions of covering. Shade may depend on tree vigour and the measurement is difficult to standardize.

In our experiment we observed that Ψstem was not significantly different following covering of 1- or 2-hours duration (Fig. 1) and variability was not affected (Table 2). In a first experiment, Begg & Turner (1970) covered leaves in the afternoon before the measurement, but in other experiments they observed that time to equilibrate Ψstem with soil water availability was lower. Chone et al. (2001) also observed that covering vine leaves for 1, 2 or 6 hours did not affect Ψstem. On the other hand, in our work Ψstem of covered leaves located in shade were significantly lower (by 0.41 MPa) than covered leaves located in the sunny exposed part of the canopy (Table 3) and variability was slightly lower (Table 2). The maximum difference of 34% were reached under conditions of high hydration (Fig. 2-B). These results have not been previously reported and indicate that covering leaves with aluminium doesn't completely stop transpiration and the leaves covered but located in the sun continue losing water specially when are highly hydrated. The higher temperature at the sun can play an important role. Our results indicate that shoots should be located in the shade and must be covered 1 hour before measurement. CV was reduced in 0.08% compared with covered leaves but located in the sun.

Ψstem also responded negatively to height of measurement within the hedgerow. This is a response to a gradient of Ψstem established in the central trunk that is related to the distance from the roots (Fig. 3). The average values were -2.06, -2.13 and -2.30 MPa for Ψstem measured at H0, H1 and H2, respectively. This form of response

Table 4. Experiment 4: Differences in midday stem water potential (Ψstem, MPa) measured in leaves of different ages (1, 2 and 3 yr old) located in the shade and middle part of the hedgerow (0.85-1.70 m) and covered 1 hour. CV = coefficient of variation (%), standard error (SE) and statistical p-value. Number of repetitions: n=10.

Day of measurement	Days since last irrigation	1 yr old			2 yrs old			3 yrs old			p-value
		Ψstem	CV	SE	Ψstem	CV	SE	Ψstem	CV	SE	
19/07/2017	1	-1.52	4	0.02	-1.49	5	0.02	-1.54	4	0.02	0.32
21/07/2017	3	-2.18	6	0.05	-2.21	7	0.06	-2.23	10	0.08	0.91
24/07/2017	6	-2.89	4	0.05	-2.90	4	0.04	-2.90	3	0.03	0.97
26/07/2017	8	-3.47	3	0.03	-3.44	2	0.03	-3.48	2	0.02	0.57
28/07/2017	10	-4.00	2	0.03	-3.98	3	0.05	-3.93	2	0.03	0.34

was also observed by Begg & Turner (1970) and Connor et al. (1977), who studied the decreasing vertical gradient of Ψstem in a small tobacco plant and a tall mountain ash forest, respectively. In contrast, in the more homogeneous canopy of a grapevine trellis Chone et al. (2001) observed similar Ψstem on basal leaves of vines with those leaves 1 m above. These Ψstem differences with height position in olive are explicable by the high resistance to water movement observed in olive trees resulting from small diameter (40 µm) of xylem vessels (Fernández et al., 2006) compared to the larger diameter of vines (75 µm) (Pire et al., 2007). Bongi et al. (1994) also attributed the differences of Ψleaf and Ψroot in olive to the high resistance to water movement. In our study, differences in Ψstem along hedgerow depend on the height; a reduction of 0.10 MPa/m was observed measuring at 0.6 m or 1.3 m, but larger differences were observed between 1.3 and 1.9 m (-0.33 MPa/m). Our results point out for the first time the importance of the position on olive hedgerow of the selected leaves in order to use Ψstem threshold values and middle position on the hedgerow (0.85-1.70 m) or slightly lower should be recommended. Several authors have used mid-height measurements to determine tree Ψstem (Marra et al., 2016; Ahumada-Orellana et al., 2017), that could be the recommended position of the selected leaves in order to compare data.

Olive is a perennial tree and leaves remain active during 2-3 years and have differences in morphological and physiological aspects (Fernández et al., 1997). Our experimental results indicate that Ψstem was not affected by the leaf age (1, 2 or 3 yr old) (Table 4). The hydration equilibrium between apex shoots and stem is achieved in leaves of different ages indicating similar water resistance and so the strength of this method to estimate the xylem sap pressure independently of the physiology of the specific measured leaves (Begg & Turner, 1970). Meanwhile, we observed lower variability of Ψstem measured in the youth leaves (CV was reduced in 0.65%) and recommend to use these leaves for better data (Table 2).

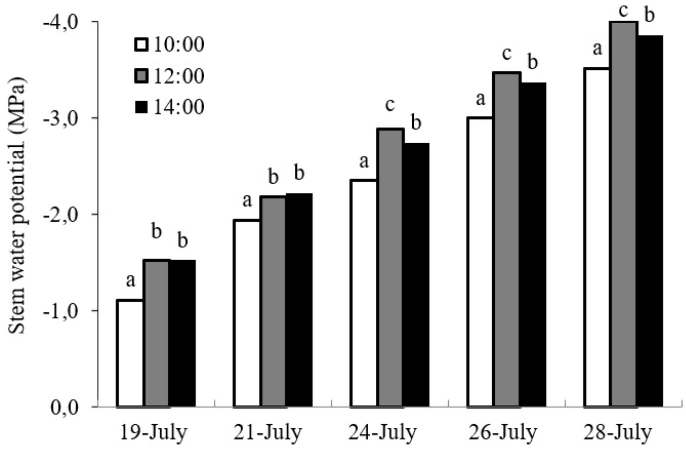

Figure 4. Experiment 5: Differences in stem water potential measured at three hours of the day (10:00, 12:00 and 14:00, solar time) in leaves previously covered for 1 hour and located in the shade and middle part of the hedgerow (0.85-1.70 m). Last irrigation was applied the 18th July 2017. Different letters indicate significant differences (p≤005) within measured hours according to the LSD test.

Table 5. Experiment 6: Midday stem water potential (Ψstem, MPa), statistical optimal sample size, coefficient of variation (CV, %) and standard error (n=15) measured in three irrigation sectors of a hedgerow orchard. Leaves were located in the shade and middle part of the hedgerow (0.85-1.70 m) and covered during 1 hour.

	Sector 1				**Sector 2**				**Sector 3**			
	Ψstem	**Sample size**	**CV**	**SE**	**Ψstem**	**Sample size**	**CV**	**SE**	**Ψstem**	**Sample size**	**CV**	**SE**
31/07/2018	-2.30	3	4	0.02	-2.37	5	5	0.03	-2.36	6	5	0.04
02/08/2018	-2.90	4	4	0.03	-3.14	6	4	0.03	-3.31	4	3	0.03
04/08/2018	-3.57	5	3	0.03	-3.64	3	3	0.02	-3.63	4	3	0.03
06/08/2018	-4.02	8	4	0.04	-4.19	7	3	0.03	-4.09	6	3	0.03
08/08/2018	-4.64	4	2	0.03	-4.80	5	2	0.03	-4.85	5	2	0.03
Average	-3.48	5	3	0.03	-3.63	5	3	0.03	-3.65	5	3	0.03

Ψstem responded to the time of measurement when made 2 hours earlier or later than noon (Fig. 4). The highest values (mean value -2.38 MPa) were achieved in the morning (10:00 solar time) and the lowest at noon (-2.81 MPa) while those of the afternoon (14:00) revealed a slow recovery to -2.74 MPa. Our results confirm that Ψstem varies depending on time of measurements and the most effective time is solar noon, as used by other authors (Fernández et al. 2011; Gomez-del-Campo, 2013; Padilla-Díaz et al., 2016; Gucci et al., 2019; Hueso et al., 2019; Trentacoste et al., 2019). The measurements made here are also consistent with the study of Fernández & Moreno (1999), which also reports that the most stable and minimum Ψstem values are obtained at solar noon, but our result indicate larger differences with previous hours that when measured later (Fig. 4).

Soil spatial characteristics in olive orchards is a source of variability in Ψstem because they determine root growth and water availability for the tree. We have observed that 5 samples of different trees provide a representative value of Ψstem for individual and soil homogeneous irrigation sectors with a variation of ± 0.17 MPa (Table 5). In a similar study in apple, Naor et al. (2006) established that a larger sample size of 7 was required for a representative value, in that case with a mean variation of ± 0.15 MPa. Fulton et al. (2001) recommended in fruit trees to sample 10 leaves when the time of equilibration is reduced to 10 min. Our results are between the minimum values reported by Naor et al. (2006) 5-15 depending on the inherent variability of the irrigation sector. In this sense, remote sensing equipment can aid in mapping tree water status (Berni et al., 2009) and help determining the representative site of the sector to be sampled, what could help to reduce sampling size, and therefore the cost of irrigation management. The measurement of Ψstem should be made as quickly as possible around noon since, as discussed above, it increases and then decreases as noon arrives and is past.

In our experiments, the standard errors were between 0.05 and 0.10 MPa, and CV between 2.7 and 4.4%, although all measurements were done by the same operator reducing variability source (Levin, 2019). Higher variability was obtained when Ψstem was measured under low stress conditions (Tables 3, 4 and 5), indicating homogeneous tree hydration level under very dry conditions (< -3.5 MPa). This response differed from other experiments (Shackel et al., 1997; Naor et al., 2006) where deficit conditions were imposed at Ψstem higher than -1.8 MPa. The most important reduction of variability was observed sampling youth shoots located in the shade, then CV could be reduced in nearly 2% (Table 2) compared with covering shoots in the sun.

Finally, Ψstem was proposed as the standard measurement to determine tree water status (Begg & Turner, 1970; Shackel et al., 1997; Naor et al., 2001) but care should be taken in the methodology used. Our results indicate the lower variability of the measurement when sampling 1-yr-old leaves located in the shadow and previously covered for 1 hour (CV=3.6%), by a minimum sample size of 5 representative leaves.

Conclusions

Ψstem measures tree-water status but there is currently no defined methodology for its measurement and hence determination of threshold values required for irrigation management. In order to reduce variability and compared values between experiments and determine threshold values, methodology should be carefully considered. In this work we firstly reported some aspects related with the particular olive orchard characteristics: covering leaves with aluminium doesn't completely stop transpiration, Ψstem depends on leaf height on olive hedgerow, Ψstem of youth leaves was less variable than the older ones and Ψstem at solar noon presented larger differences with previous hours than later.

Our results indicate that sampled leaves should be located at mid height of the hedgerow and should be covered with aluminium bags before Ψ measurement even when they are located in the shade in order to reduce transpiration and variability. It is sufficient to cover shoots for 1 hour but they may remain covered for 2 hours without effect on measurement. Ψstem values depend on time of measurement but not on age of leaves. It is recommended that measurements are made at solar noon but lower differences are observed when the measurement is delayed in time rather than advanced. Leaves should be 1 year old, because the variability is lower than in 2- or 3-year old leaves, but Ψstem was not significantly affected by leaf age. Considering these methodological recommendations, CV of Ψstem can be reduced in more than 2%. Special care must be taken under high hydration conditions because measurement methodology has more influence on results than under water-stress conditions.

Under the studied conditions, to obtain a representative value of midday Ψstem of a soil-homogeneous irrigation sector within an orchard, it is required to take measurements on 5 shoots of 5 different trees applying the practices presented above.

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